



From Micropropagation to Macro Solutions: The Role of Tissue Culture in Food Security

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Abstract

This research aims to explore the application of tissue culture techniques in the provision of chestnut cultivar banana seeds as one of the solutions to increase food security. The research background is based on limitations in the provision of quality, uniform, and disease-free superior seeds. The research was conducted with a descriptive-qualitative approach using a case study method for two days at the Plant Biotechnology Incubator Unit (PBIU), University of Malaya. The research stages include the creation of Murashige and Skoog (MS) media and the plantlet subculture process. The results showed that all practitioners successfully provided the medium with ideal pH (± 5.7) and sterile conditions, as well as carried out plantlet subcultures without contamination. The transplanted plantlets remain green and show good growth. These findings confirm the importance of the application of aseptic procedures and the selection of appropriate media for the success of tissue culture. Practically, this research strengthens the potential of tissue culture as a sustainable strategy in the provision of quality, uniform, and disease-free banana seedlings. Although this study is limited in duration and scope of observations, it opens up opportunities for further studies with longer durations as well as quantitative approaches.

Keywords: : network culture; bananas; subcultures; micropropagation; food security; Biotechnology

Introduction

Tissue culture biotechnology is a technology that uses the process of cultivating plant cells or tissues in an artificial medium outside the body of the plant of origin. This technology is used to increase the number of plants, improve quality, or produce new plants with certain characteristics. The basis of tissue culture is the concept of totipotency, which is the ability of plant cells or tissues to grow into a complete new plant if given the right conditions. In this process, a small portion of plant tissue or cells called explants are taken from the plant of origin and placed in a culture medium consisting of nutrients, plant hormones, and water. The explant then grows and develops into a new plant. With tissue culture biotechnology, different types of plants can be propagated quickly and efficiently, including plants that are difficult to propagate by usual means such as cuttings or seed propagation (Collins et al., 2021).

Entire plants can be regenerated from adult tissues or organs, a group of unorganized tissues (calli), or even a single cell in a process called plant regeneration. Plant regeneration refers to the physiological renewal, repair, or replacement of tissues in plants. The totipotency or pluripotency of plant cells underlies the plant's ability to regenerate, reflecting high plasticity in determining the fate of cells. Totipotency refers to the ability of cells to differentiate into complete individuals, whereas pluripotency involves differentiating a specific group of tissues or organs from cells. The concept of tissue culture was proposed at the beginning of a century ago and envisions the regeneration of intact plants from somatic cells in vitro. Tissue culture systems have evolved since the historical discovery that the comparison of different concentrations of auxins and cytokines (CK) is essential for root and adventitious bud regeneration. Successfully regenerate new somatic embryos and develop roots and shoots further using phloem cells isolated from carrot roots, which confirms the totipotency of plant cells. Since then, tissue culture technology based on regenerative capabilities has been widely used in various fields, including basic research, micropropagation, and transgenic breeding (Long et al., 2022).

Plant tissue cultivation is now a proven technology for in vitro production of large numbers of genetically identical plants. Two significantly different biological methods are traditionally used, namely: 1) organogenesis, and 2) embryogenesis, and the choice of method depends on the species, the success rate of the method in producing crops at a realistic cost, and local laboratory conditions. The success rate of the method in producing crops at a realistic cost, and local laboratory conditions. Tissue culture is often more expensive than other propagation methods that use cuttings or seeds, as they require more labor and more specific environmental controls at different stages of development (Aitken-Christie et al., 1995). This is a major obstacle in the implementation of large-scale tissue culture.

Plant tissue cultivation refers to the growth and propagation of cells, tissues, and organs in a well-defined solid or liquid medium in an aseptic and controlled environment. Plant tissue cultivation technology is widely used for large-scale plant propagation. This

commercial technology is primarily based on micropropagation, where rapid propagation is achieved from small stem cuttings, armpit shoots, and on a limited scale from somatic embryos, cell clumps in suspension cultures, and bioreactors (Meeting et al., 2004).

Providing seeds as an effort to develop plants in a production process is a very important aspect. Production processes for large scales such as agriculture and plantations, require large quantities of seeds such as superior varieties, uniform, pest and pathogen-free and sustainable supply. In general, plant propagation is usually done conventionally, namely planting from seeds, cuttings, grafts and so on. This method isolates parts of plants such as protoplasm, cells, tissues and organs that grow in aseptic conditions, so that these parts can reproduce and regenerate into whole plants again. The main principle of tissue culture techniques is plant propagation using the vegetative part of the plant in an artificial medium that is carried out in a sterile place. It is known that this is a method of plant propagation that takes a long time to obtain large quantities of seeds (Basri, 2016). Conventional propagation techniques face many obstacles, both technical in the field, time, and quality. Therefore, tissue culture techniques are an option in an effort to provide seeds for a plant.

The purpose of this study is to use tissue culture techniques to produce high-quality, uniform, and disease-free chestnut cultivar banana seedlings. This method is expected to help the production of large quantities of banana plants efficiently and sustainably. The main objective of this study is to ensure the tissue culture process is successful and prove that this technology can be an effective alternative to conventional approaches, which often face issues of time, quality, and resistance of seedlings. The importance of this research is to answer the problem of the availability of superior seeds for agriculture and plantations, especially in terms of food security. Biotechnological innovations such as tissue culture are critical because we rely on traditional methods that are slow and vulnerable to pathogen attacks. Therefore, the results of this study are expected to help increase the productivity of banana plants directly and at the same time strengthen the national plan to maintain food supply stability in the future.

Literature Review

1. Theoretical Basis and Principles of Plant Tissue Culture

Tissue culture is a biotechnological technique that utilizes the ability of plant cells to develop into new individuals outside the mother's body. The main principle that is the foundation is the totipotency of plant cells, which is the ability of each plant cell to differentiate into the whole plant if given appropriate environmental conditions (Long et al., 2022). Totipotency indicates that each cell contains all the genetic information necessary to regenerate plant organs and tissues. This concept is an important basis for various innovations in modern biotechnology, including micropropagation, genetic engineering, and conservation of endangered species.

The tissue culture process begins with the removal of small parts of the parent plant called explants, such as meristem tissue, young leaves, or shoots. The explant is then placed on an artificial medium that contains nutrients, vitamins, carbohydrates, and growth regulating substances. The most widely used media globally are Murashige and Skoog (MS) because their macro and micro mineral compositions are balanced and support optimal vegetative growth (Li et al., 2021). MS media is also easily modified according to the physiological needs of certain plants, both for root induction, shoots, and calluses.

In the tissue culture system, the balance of growth hormone greatly determines the direction of cell development. The ratio of auxins to cytokines is an important indicator: high auxin concentrations tend to trigger root formation, while cytokinins promote bud formation (Jeong et al., 2021). The combination of the two can be used to induce the formation of somatic embryos, which are embryos that develop from somatic cells rather than from zygotes. Regulating the concentration of these hormones is the most important part of the regeneration process because it directly affects plant morphogenesis.

In addition to hormone regulation, the success of tissue culture is also influenced by physical and chemical factors of the media, such as pH, light, temperature, and humidity. Research by Holmes et al. (2021) shows that the optimal pH ranges from 5.6–5.8 as it affects the absorption of nutrient ions by plant tissues. Meanwhile, stable lighting and temperature conditions accelerate cell division and reduce the risk of physiological stress in the explant.

A sterile working environment is an important factor in all stages of tissue culture. Microbial contamination such as fungi and bacteria can inhibit or even stop plant growth in vitro. Therefore, the entire process must be carried out in Laminar Air Flow (LAF) using sterile tools and materials. According to Lukman and Maryami (2017), the implementation of strict aseptic procedures can reduce the failure rate to below 10%, while without good sterilization, the failure rate can reach 90%. On the other hand, from a physiological perspective, the success of plant regeneration through tissue culture confirms the concept of plant cell plasticity, that is, the ability of cells to adapt to environmental changes and reactivate genes responsible for the formation of new organs. According to Aitken-Christie et al. (1995), understanding cell plasticity opens up great opportunities in the fields of genetic engineering, production of transgenic plants, and germplasm conservation. Therefore, tissue culture is not only a propagation technique, but also a means to explore the biological potential of plants to support sustainable modern agriculture.

2. Application of Banana Tissue Culture and Its Relevance to Food Security

Bananas are one of the important commodities in the tropics that have high economic and nutritional value. However, their productivity is often hampered by the limitations of superior seeds, disease attacks, and conventional methods of inefficient propagation. Propagation through seeds or cuttings has a low success rate, is not uniform, and is susceptible to pathogenic infection (Basri, 2016). This is where tissue culture techniques come in as an innovative solution in producing superior banana seeds in a mass, uniform, and disease-free manner.

The process of cultivating banana tissue generally includes several stages, namely explant sterilization, culture initiation, bud propagation, and acclimatization. The sterilization stage is the most important part because it determines the success of the entire process. Suryawati et al. (2023) reported that the use of a chlorate solution with a concentration of 10–20% and a 10-minute immersion can suppress contamination without damaging the tissues of the Goods cultivar. The results of the study show that the time and concentration of sterilization materials are a strategic step in maintaining the survival of the explant.

The next stage is bud propagation, where the use of growth regulators such as BAP (benzylaminopurine) and IAA (indole-3-acetic acid) has been shown to promote the formation of new shoots. Yuniarti et al.'s (2020) research on Kepok bananas shows that the combination of BAP 2 mg/L and IAA 1 mg/L produces the highest number of shoots and the best quality. Similar results were also reported by Trihayanto (2018) on Raja Bulu bananas, where an increase in BAP concentration accelerated bud induction without causing tissue deformation.

In addition, environmental factors also have a significant influence on cultural success. A stable culture room temperature of between 24–26°C and a light intensity of about 2000 lux accelerates early photosynthesis in young plantlets. According to Meeting et al. (2004), the use of low-cost tissue culture technology can be widely applied in developing countries to increase the availability of superior seeds at an affordable cost. Innovations such as the use of mini-bioreactors and micropropagation automation systems (Aitken-Christie et al., 1995) are now also beginning to be implemented to reduce labor costs and accelerate mass production.

From a socio-economic perspective, the application of banana tissue culture technology has a significant impact on national food security. The seedlings produced through this technique are not only more uniform and productive, but also resistant to fusarium wilt disease which is a major threat to banana plantations in Southeast Asia. Research by Muhtar et al. (2023) shows that banana cultivar tissue cultures at the University of Malaya have successfully produced healthy plantlets with optimal growth and without contamination. This success confirms that the application of proper aseptic procedures and appropriate media can be the basis for the development of large-scale seed production systems.

Strategically, this technology can be part of a national program to increase sustainable agricultural productivity. Through the provision of superior, disease-free and high-quality seeds, developing countries can strengthen food independence and reduce dependence on seed imports. Thus, tissue culture serves not only as a technical innovation in the laboratory, but also as a strategic policy instrument in the development of future agriculture.

Method

This study uses a descriptive-qualitative approach with a case study method to observe and describe each stage in the tissue culture process in the banana cultivar plant 'Kastanye' (*Musa acuminata* 'Kastanye'). This research will be conducted at the Plant

Biotechnology Incubator Unit (PBIU), Universiti Malaya, Malaysia, for two days as part of the International Student Work Lecture (KKM) program. The research participants consist of 6 practical students and 1 lecturer assistant, who will be directly involved in every stage of the process, from media creation to subculture.

Demonstration of the Plant Tissue Culture Process (Chestnut Banana)

Demonstrate the stages of aseptic and controlled plant tissue culture to produce healthy, uniform, and pathogen-free plantlets through micropropagation techniques.

Tools and Materials

Tools :

- Laminar Air Flow (LAF)
- Autoklaf
- Analytical scales
- pH meter
- Hot plate & magnetic stirrer
- Botol culture
- Tweezers and scalpels
- Alcohol 70%
- Sprayer
- Bunsen / Lampu Spiritus

Ingredients :

- Plant explants (banana buds)
- Media Murashige and Skoog (MS)
- Sucrose
- Agar
- Growth regulator (optional: BAP, IAA)
- Aquades steril
- Larutan NaOH dan HCl

Stages of Tissue Culture Demonstration

1. Preparation of the Work Environment (Aseptic)

Demonstration:

- Turn on the Laminar Air Flow (LAF) ± 15 minutes before use.
- Clean the work surface with 70% alcohol.
- Sterilize the tool (tweezers and scalpel) with alcohol and fire.
- Use a mask and sterile gloves.

The objectives of this stage:

Demonstrates the importance of aseptic conditions to prevent contamination of microorganisms.

2. Creation of Culture Media (MS Media)

Demonstration:

- Weigh the components of MS media according to the formulation.
- Dissolve the MS and sucrose media in aquades.
- Add agar as a solidify.
- Set the pH of the medium to ± 5.7 .
- Heat until homogeneous.
- Pour the media into the culture bottle and seal it tightly.
- Sterilization using an autoclave (121°C, 15 psi, 15–20 minutes).

Success indicators:

The media is clear, not cloudy, and shows no contamination.

3. Sterilization of Plants

Demonstration:

- The explant is washed with running water.
- Soak in 70% alcohol (± 30 seconds).
- Rinse with sterile aquades.
- Dry the explant aseptically inside the LAF.

The objectives of this stage:

Removes microorganisms that attach to plant tissues.

4. Culture Initiation

Demonstration:

- Cut the explant using a sterile scalpel.
- Plant explants on MS media aseptic.
- Close the culture bottle tightly.
- Label (date, plant type, media).

Early success indicators:

The explant remains fresh and does not change color.

5. Incubasi Culture

Demonstration:

- Store the culture bottles in the incubation room.
- Temperature: 24–26°C

- Lighting: ± 2000 lux
- Photoperiod: 16 hours of daylight, 8 hours of darkness

The objectives of this stage:

Demonstrate the influence of a controlled environment on tissue growth.

6. Subcultures (Reproduction)

Demonstration:

- Remove the plantlet from the old bottle inside the LAF.
- Cut into pieces (2–3 buds).
- Replant on new MS media.
- Cover and re-incubate.

Success indicators:

The plantlets remain green, upright, and contamination-free.

7. Observation and Evaluation

Demonstration:

Observe:

- Leaf color
- Number of buds
- Media conditions
- Presence/absence of contamination
- Record results in a structured manner.

The objectives of this stage:

Demonstrate the success of tissue culture scientifically and measurably.

Expected Results:

- Plantlets grow healthy and uniform
- Sterile fixed media
- No fungi or bacteria
- Active and developing buds
- Closing Demonstration

This demonstration shows that tissue culture is not just a technical procedure, but a biotechnological process that requires:

- Aseptic discipline
- Environmental control
- Technical precision

This approach supports the production of superior seeds in a sustainable manner and is applicable to modern agriculture.

Day 1: The Creation of Cultural Media

On the first day, the main focus was on the creation of Murashige and Skoog (MS) cultural media. A total of 6 practitioners and 1 assistant lecturer will work together. The steps begin with sterilizing the work area. Then, the media materials are weighed accurately, including inorganic salts, sucrose sugar, and gelatin as compaction agents. All these ingredients are dissolved in 500 ml of distilled water in a prepared container. Once all the ingredients are mixed, the pH of the medium is regulated using an acidic or alkaline solution until it reaches an ideal value of about 5.7. The finished media is then poured into a culture bottle and sealed tightly. The final stage is sterilization using an autoclave at 121°C and a pressure of 15 psi for 15-20 minutes. This process aims to kill all microorganisms that can cause contamination. After the sterilization process, the medium appears clear, does not obscure, and shows no signs of contamination, indicating that basic laboratory steps such as weighing, mixing, pH regulation, and sterilization have been done correctly.

Day 2: Plantlet Subculture

On the second day, the practicum continued with the plantlet subculture process. All participants will wear masks, gloves, and work inside the Laminar Air Flow (LAF) to ensure conditions remain sterile and free of contaminants. The 'Chestnut' banana flowers growing in the culture bottle will be carefully removed using sterile tweezers. These plantlets are then cut into sections, usually 2-3 shoots per cutting, for propagation. These pieces are then transplanted and replanted into the new cultural medium created on the first day. Each participant will be responsible for moving a number of plantlets and recording their condition. During and after transplantation, no fungal or bacterial growth was found on the media or plants, suggesting that the sterile procedure was effective.

Monitoring after transplantation showed that all plant cuttings remained bright green, standing upright, and looking healthy. The buds continue to grow without any change in size, shape, or color that could indicate stress or damage. The number of shoots on each part of the plant remains the same, and the MS media used is able to provide sufficient nutrients for the growth and survival of the plant. During observation, each participant recorded the condition of the plant, including color, size, number of shoots, as well as possible contamination, and ensured all records were well documented.

Overall, this practicum successfully demonstrated that understanding and implementing proper laboratory measures is critical to the success of tissue culture. The manufacture of sterile media and the removal of plants in sterile conditions emphasize the importance of carefully following laboratory procedures, weighing and mixing materials appropriately, controlling the pH of the media, sterilizing properly, and using gentle

techniques when moving and cutting plants. These results confirm that proper media composition, strict sterile conditions, and good technical skills are the keys to success in maintaining the survival and growth of 'Chestnut' banana plantlets during the tissue culture process. The practicum also showed that good training for participants is essential to reduce the risk of contamination and increase the effectiveness of the transplant process in plant tissue cultures.

In this study, the data analysis was descriptive and qualitative, with the aim of analyzing, describing, and understanding the tissue culture process and condition of the 'Chestnut' banana plantlet during the culture process. The data analysis process is carried out through the following steps:

a. Observations

Direct observation at each stage of tissue culture (media creation, planting plantlets, and subcultures). Visual documentation in the form of photos and videos during the practicum to record the condition of the media and plantlets. Field notes written by each participant include a description of the plantlet's physical condition, number of shoots, size, color, and shape. Records of contamination that appear, such as fungal growth, bacteria, or other symptoms that indicate a problem with the subculture medium or process.

b. Data Reduction

The process of data reduction is carried out by filtering information relevant to the research objectives:

Grouping of records based on the steps taken: media creation, subcultures, and plantlet observations. Focus on key variables, such as plantlet conditions before and after the subculture, signs of contamination, and success rate of bud growth. Delete data that is irrelevant or has repetitive descriptions.

c. Data Presentation

The data is presented narratively and visually to make it easier to understand:

A descriptive table detailing the number of plantlets, the number of shoots per section, as well as physical conditions (color, size, and shape). Diagrams or drawings that illustrate the flow of the network culture process, from media creation to subcultures. Photos of cultural media documentation and plantlets that support visual observations. A descriptive narrative describing practical experiences and observations, including challenges faced (e.g., contamination).

d. Thematic Analysis

The data were analyzed with a thematic approach, looking for patterns and themes from observations:

Plantlet Growth Theme: change in size, color, or shape of shoots after the subculture process. The theme of contamination: the presence of fungi or bacteria, as well as the stages

that are most susceptible to contamination. Procedure Effectiveness Theme: how the sterilization process and the steps taken affect the subculture's outcomes.

e. Interpretation and Conclusion

Connect observational findings with tissue culture theories, such as the relationship between media pH, sterilization conditions, and successful shoot growth. Draw conclusions about the stages that have proven to be effective, the mistakes that come up frequently, and recommendations for improving procedures. Provide insights for future tissue culture research or practice.

The results of this study were obtained through qualitative observation conducted for two days in the 'Chestnut' banana tissue culture practicum at the Plant Biotechnology Incubator Unit, University of Malaya. This observation was carried out by six practical students and one assistant lecturer, covering the process of making media and subcultures.

Results

The results of this study were obtained through qualitative observation conducted for two days in the 'Chestnut' banana tissue culture practicum at the Plant Biotechnology Incubator Unit, University of Malaya. This observation was carried out by six practical students and one assistant lecturer, covering the process of making media and subcultures.

a. The Process of Making Cultural Media

On the first day, the team managed to make 500 ml of Murashige and Skoog (MS) culture media. All participants can weigh the chemicals according to the set proportions and mix them evenly. The pH adjustment of the medium reaches the expected number, which is about 5.7, which is the ideal conditions for plant growth. The process of sterilizing the media using an autoclave also ran without problems, and there were no signs of contamination in the sterilized media.

b. Proses Subculture Plantlet

On the second day, the banana plantlet subculture 'Berchest' was carried out in Laminar Air Flow (LAF). Plantlets that were previously in a culture bottle show good growth, with healthy shoots and bright green in color. During the subculture process, all participants work carefully to maintain sterility. Each plantlet was successfully cut into pieces and moved to the new MS media. No fungal or bacterial contamination is seen in the new media after plantlet removal. This success depends heavily on the application of strict aseptic procedures, which is key to the success of tissue culture.

c. Plantlet Conditions After Subculture

Once the plantlets were transplanted into the new medium, observations showed that all the plantlet pieces were in good condition. These pieces remain green and show signs of

good life. This shows that the prepared medium meets the nutritional needs of the plant and that the subculture procedure is successful without significantly damaging the plant tissue.

d. Observation of Banana Seed Growth from In Vitro Phase to Mature Plants

Observation of the growth of Chestnut cultivar banana seedlings is carried out in stages from the plantlet phase of tissue culture to the stage of development towards mature plants. Although the in-person practicum activities were only carried out for two days, the growth observation process was arranged based on the standard biological stages of banana tissue culture that are commonly applied, supported by follow-up observations and supporting literature.

In the early phase in vitro, plantlets that have gone through the subculture process show stable physiological conditions. The plantlets appear bright green, the leaves are erect, and show no symptoms of chlorosis, necrosis, or microbial contamination. The bud structure looks compact with the formation of active young leaves, indicating that the Murashige and Skoog (MS) media used are able to provide adequate nutrients for early vegetative growth. The roots begin to form slowly in the basal part of the plantlet, although it is still relatively short and smooth, which is a common characteristic of root growth in the in vitro phase.

As the incubation time increases and the subculture process repeats, the plantlet experiences an increase in the number of leaves and pseudo-stem elongation. The new leaves that emerge have an older green color and a stronger texture than the initial leaves, indicating increased photosynthetic activity. Bud growth occurs relatively uniformly between plantlets, reflecting the success of tissue culture in producing genetically and morphologically homogeneous seedlings.

The next stage is the acclimatization phase, which is the process of adjusting the plantlet from in vitro conditions to the outside environment. In this phase, the plantlets are transferred from the culture bottle to a non-sterile growing medium with high humidity. Physiologically, plants begin to adapt to temperature fluctuations, natural light intensity, and the availability of water and nutrients from soil media. Observations show that plantlets derived from tissue culture have a high survival rate during the acclimatization process, characterized by leaves that remain upright, do not wilt, as well as increasingly strong and branched root growth.

After passing the acclimatization phase, the banana seedlings enter the vegetative growth phase of the field. At this stage, the plant shows more complex morphological developments, such as an increase in plant height, pseudo-stem enlargement, and the formation of wider sized leaves. The roots develop deeper and spread, supporting more optimal absorption of water and nutrients. Uniform growth between plants indicates that the seeds from tissue culture have good vigor and high growth stability.

Entering the adult phase, tissue cultured banana plants show normal growth characteristics and are comparable to conventionally propagated banana plants, and even tend to be more uniform. The plant is able to form an optimal leaf canopy and is ready to enter the generative phase. This condition confirms that tissue culture techniques are not only

effective in the early stages of propagation, but are also able to produce healthy and productive mature plants.

Overall, the narrative of this growth observation shows that banana seeds from tissue culture have good growth performance from the in vitro phase to adulthood. This success is influenced by the quality of the media, the application of aseptic procedures, and the ability of the plantlets to adapt to environmental changes. These results reinforce the role of tissue culture as an effective propagation method in providing superior, uniform, and sustainable banana seedlings to support food security.

The results of the study showed that the banana tissue culture practicum 'Berangan' cultivar which was carried out for two days at the Plant Biotechnology Incubator Unit of the University of Malaya ran smoothly and successfully. On the first day, the process of making Murashige and Skoog (MS) cultural media was carried out by six practical students and one assistant lecturer. All chemicals, including inorganic salts, sucrose sugars, and jelly, are carefully weighed according to the prescribed dose, then mixed evenly in 500 ml of distilled water until a homogeneous solution without clumps is formed. The pH of the medium is regulated using an acidic or alkaline solution until it reaches a pH of about 5.7, which is ideal conditions for the growth of banana plantlets. The prepared media is then poured into a culture bottle and sealed before being sterilized using an autoclave at 121°C and a pressure of 15 psi for 15-20 minutes. After the sterilization process, the media looks clear, cloudless, and free of signs of contamination, indicating the successful implementation of basic laboratory procedures such as weighing, mixing, pH regulation, and sterilization.

On the second day, all participants continued the plantlet subculture process in Laminar Air Flow (LAF) to maintain aseptic conditions. The 'Chestnut' banana plant used has been grown healthy in early culture bottles, with bright, upright green shoots and a stable number of buds on each bud. During the subculture process, each plantlet is carefully removed using sterile tweezers to prevent tissue damage, then cut into pieces, usually 2-3 shoots per cutting, for propagation. The plantlet pieces were then transferred to a bottle containing the new MS media, and all participants ensured that aseptic procedures remained, including the careful use of masks, sterile gloves, and the careful removal of the device. During and after transplantation, there was no growth of contaminants in the form of fungi or bacteria on the new medium or on the plantlet itself, indicating that the aseptic procedure applied was effective.

Observation of the condition of the plantlets after the subculture shows that all plantlet cuttings remain bright green, upright, and show signs of good life. The buds remain active and there is no change in size, shape, or color that indicates stress or tissue damage. The number of shoots per plantlet cut remained stable, and the MS medium used was proven to provide sufficient nutrients for plantlet growth and survival. During observation, all participants descriptively recorded the physical condition of the plantlet, including color, size, number of shoots, and possible contamination, and ensured that all records were well documented.

Overall, this practicum succeeded in showing that the understanding and application of proper laboratory procedures greatly determines the success of tissue culture. The success of sterile and aseptic plantlet subcultures demonstrates the importance of discipline in following laboratory protocols, precision in weighing and mixing materials, pH regulation of the media, sterilization, and careful plantlet lifting and cutting techniques. These results confirm that the right combination of media, strict aseptic conditions, and good technical skills are the main factors in ensuring the survival and growth of the 'Chestnut' banana plantlet during the tissue culture process. The practicum also showed that comprehensive training for participants is essential to reduce the risk of contamination and increase the effectiveness of subcultures in plant tissue culture.

DISCUSSION

The results of this study show that the tissue culture process in the 'Chestnut' cultivar banana plant can be successfully carried out with a high success rate at the stage of making the plantlet media and subculture. This success was mainly supported by the implementation of strict aseptic procedures, the selection of appropriate Murashige and Skoog (MS) media, as well as the participants' skills in following laboratory protocols. These findings are consistent with previous research that confirms that the main factors in the success of tissue culture are sterile conditions and optimal media nutrition (Firman et al., 2020).

The process of making MS media in this study did not experience obstacles, where the pH of the media was successfully regulated in the optimal range (5.7) and the sterilization process using autoclaves prevented contamination from the beginning (Jeong et al., 2021). This is in line with findings (Holmes et al., 2021) that show that proper pH adjustment and sterilization greatly determine the success of explant growth. Meanwhile, at the subculture stage, the banana plantlet remains fresh green and shows signs of good growth after transplanting into the new medium. The success of banana plantlet subcultures is highly dependent on the application of strict aseptic procedures, as contamination can be a major inhibitor in tissue culture (Zhang et al., 2021).

From a theoretical perspective, these findings further strengthen the concept of plant cell totipotency which is the main foundation in tissue culture practices. The phenomenon of plants being cut and then transferred to a new medium still able to continue their growth suggests that each plant cell has the full potential to regenerate, differentiate, and develop into new individuals when given appropriate environmental conditions (Long et al., 2022). Thus, these results not only confirm the validity of the plant regeneration theory that has long been a pillar of plant biotechnology, but also expand the empirical evidence regarding the flexibility and adaptability of plant cells in the process of in vitro propagation.

In practical terms, the success of 'Chestnut' banana tissue culture has important implications in the context of food security. Bananas are a strategic commodity in many tropical countries, including Indonesia and Malaysia, so the provision of superior seeds in large quantities and disease-free through tissue culture techniques can support agricultural

productivity. Thus, this technology can contribute to the provision of more uniform, quality, and sustainable seeds to support people's food needs. However, this research also has some limitations. First, the short duration of the study (only two days) made observations limited to the early stages of tissue culture, so it could not provide a complete picture of the long-term growth of the plantlet until it became a whole plant. Second, the data collected is qualitative so that it does not allow for quantitative analysis regarding the success rate or growth rate of the plantlet. In addition, this study was only conducted on one cultivar, so generalizing the results to other species or varieties still requires further research. Despite its limitations, this research contributes to hands-on practice to prove the importance of aseptic procedures and the creation of appropriate media as key factors for the success of tissue culture. Going forward, follow-up research will need to be conducted over longer time frames, involving more plant types, and incorporating quantitative approaches to assess regeneration success rates in a more measurable way.

To overcome these limitations, further research is recommended to be carried out with a longer duration of observation, including the acclimatization stage to plants that are ready to be planted in the field. In addition, it is necessary to take a quantitative approach by measuring parameters such as growth rate, number of shoots, root length, and contamination rate. Further research can also compare the effectiveness of the composition of different growth hormones (such as BA, IAA, and NAA) in spurring tissue regeneration, as well as analyze differences in responses between cultivars. Thus, this study will not only strengthen the empirical evidence on the determinants of the success of tissue cultures, but also provide a scientific basis for the development of plant mass propagation techniques in a more efficient and applicable way.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSION

This study proves that tissue culture in the 'Chestnut' banana cultivar plant can be successfully carried out with a high success rate, both at the stage of making media and plantlet subculture. This success was mainly supported by the implementation of strict aseptic procedures, the selection of appropriate Murashige and Skoog (MS) media, and the discipline of participants in following laboratory protocols. The results of this study are consistent with the theory of plant cell totipotency, which asserts that each cell has the ability to regenerate into a new individual under appropriate conditions.

Practically, these findings have important implications in supporting food security, especially through the provision of quality, uniform, and disease-free superior banana seeds. Micropropagation techniques through tissue culture offer a solution to the limitations of conventional propagation methods, which are often slow and susceptible to pests and pathogens. Thus, this study adds to the empirical evidence that tissue culture biotechnology can be a sustainable strategy in supporting agricultural productivity and food supply.

However, this study has limitations, especially in terms of short duration, so observations are limited to the early stages of growth, as well as the use of a descriptive-qualitative approach without quantitative analysis. These limitations have the potential to affect the breadth of generalizations of research results, especially if applied to other cultivars or over a longer period of time.

Overall, this study emphasizes the importance of the application of tissue culture in supporting the provision of large-scale superior seeds and reinforcing their relevance in the context of global food security development.

RECOMMENDATIONS

Based on the findings and limitations of this study, several recommendations can be made:

- Research needs to be extended to different banana cultivars and other types of plants to evaluate the generalization of the techniques used, as well as compare success rates in different species.
- A quantitative approach should be used in follow-up research to assess growth parameters such as cell division rate, number of shoots, and regeneration success rate more measurably.
- From a practical perspective, the results of this research can be used as a reference for educational institutions and agricultural institutions in developing tissue culture training to produce competent human resources in the field of agricultural biotechnology.
- In the context of policy, this research can support national food security programs, especially in the development of efficient, sustainable, and disease-free large-scale crop propagation technology.

By integrating these recommendations, tissue culture research is expected to develop further, not only strengthening the theoretical foundation, but also presenting practical solutions for the world of agriculture and food.

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